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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: C. Frank Bennett, David J. Ecker and Phillip Dan Cook

Serial No:

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For: COMPOSITIONS AND METHODS FOR THE PULMONARY DELIVERY OF NUCLEIC ACIDS

Group Art Unit: 1635

DECLARATION UNDER 37 CFR §1.132 OF DR. C. FRANK BENNETT

Commissioner for Patents
PO Box 1450
Alexandria VA 22313-1459

Dear Examiner Bowman:

I, C. Frank Bennett, declare that:

1. I am Vice President of Research at ISIS Pharmaceuticals, Inc., 1896 Rutherford Road, Carlsbad, California 92008. I received a Bachelor of Science degree in Pharmacy from the University of New Mexico, Albuquerque, and a Doctor of Philosophy degree in Pharmacy from the Baylor College of Medicine. I have been employed at ISIS Pharmaceuticals, Inc. since 1989. I have been Vice President of Research since 1995.

2. I am a joint inventor of the subject matter described and claimed in United States Patent Application Serial No. 09/315,292, entitled "Compositions and Methods for the Pulmonary Delivery of Nucleic Acids."

3. I am personally aware of results demonstrating the efficacy of oligonucleotides containing at least one 2'-MOE modified base for the treatment of pulmonary disease using nucleic acids targeted to a number of different genes including, but not limited to, B7.1/CD80; B7.2/CD86; complement component 3a receptor 1 (C3aR); dendritic cell-specific ICAM3-grabbing nonintegrin (DC-sign); intracellular adhesion molecule (ICAM); interleukin 4 receptor-alpha (IL4Ra); p38 alpha; STAT 6; and tumor necrosis factor alpha

(TNF-alpha). The oligonucleotides were tested in a number of animal models including mouse and monkey. A subset of these results are presented below.

4. Three different mouse models of asthma were used to demonstrate the efficacy of nucleic acid therapeutics in the treatment of pulmonary disease. The acute asthma model is a prophylaxis paradigm wherein the oligonucleotide is administered prior to allergen challenge. The rechallenge model is a therapeutic treatment paradigm after local lung inflammation has occurred, wherein cytokine levels in the lung are higher and an immunological recall response is present. The chronic model is a chronic disease paradigm wherein challenge is presented aggressively by intranasal administration intermittently during treatment administration.

5. Timelines for each of the models is shown in the attached Figure 1. In each model, mice are sensitized to chicken ovalbumin (OVA) by administration of two intraperitoneal injections of the protein at day 0 and day 14. Aerosolized OVA is subsequently administered in an allergen challenge either using a nebulizer, as in the acute and rechallenge models, or by intranasal inhalation, as in the chronic model, on the days indicated. Target specific oligonucleotides and control samples (e.g. mismatch oligonucleotides or vehicle) are administered by inhalation or by intratracheal administration.

6. A number of endpoints can be assayed in these asthma models. Data for some common endpoints analyzed in the studies are shown in Table 1. The table includes the sequence of each of the oligonucleotides used with the 2'-MOE modified bases are indicated in **bold**. Different effects on different endpoints are seen both within and between targets. This is expected as the inflammatory profile for each of the models is different, as are the pathways in which the targets are involved. However, in each of the models with each of the targets an amelioration of symptoms is observed.

7. Penh is a dimensionless parameter that is a function of total pulmonary airflow in mice during the respiratory cycle of the animal. Inhibition of Penh response to induction of airflow obstruction using metacholine. The lower the Penh value, the greater the airflow. A decrease in Penh is indicative of a decrease in airway hyperresponsiveness.

7. Eosinophils and neutrophils are inflammatory cells. A decrease in infiltration of these cells is indicative of a decrease in inflammation. A decrease in the level of various cytokines in the lung was also observed (data not shown).

8. Mucus, another indicator of inflammation, occludes airways. Reduction of mucus production ameliorates disease.

9. Oligonucleotide toxicities tend to be based on overall chemistry of the oligonucleotide, rather than the specific sequence of the oligonucleotide. As a class, 2'-MOE containing oligonucleotides have been found to have a relatively low toxicity when administered by a number of routes, including inhalation.

10. Pharmacokinetic studies demonstrate that the half-life for 2'-MOE oligonucleotides administered by inhalation to mice is at least about four days.

11. These results demonstrate that an oligonucleotide containing at least one 2'-MOE modification is delivered to the lung by aerosol. The data further demonstrate that delivery of an oligonucleotide to the lung by aerosol can be used for the treatment of pulmonary disease.

12. I declare that all statements made herein are of my own knowledge true and statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

C. Frank Bennett

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5-11-05

Date

Table 1
Summary of mouse asthma model data for 2'MOE-containing oligonucleotides

Gene	Isis No	SEQ	Model	Decrease target expression	Decrease eosinophil recruitment	Decrease neutrophil recruitment	Decrease metacholine response	Decrease mucus production
B7.1/ CD80	121844	GCTCAGCCTTTCCACTTCAG	Acute	+	+	ND	--	ND
B7.2/ CD86	121874	TCAAGTTTCTCTGTGCCCAA	Acute	+	+	ND	+	ND
C3aR	260721	GCCACATCTTCACACATCTG	Chronic	ND	--	--	--	+
DC-sign	290135	CACTAACCCAAAGAGAACCT	Acute	ND	+	ND	+	ND
DC-sign	290135	CACTAACCCAAAGAGAACCT	Chronic	ND	--	--	--	+
ICAM	17481	TCCCACAGCAGCTTGCACGA	Chronic	ND	ND	ND	ND	+
IL4R alpha	231894	CCGCTGTTCTCAGGTGACAT	Acute	ND	+	ND	+	ND
IL4R alpha	231894	CCGCTGTTCTCAGGTGACAT	Rechal.	ND	+	+	+	+ ($p \leq 0.07$)
p38	101757	AGGTGCTCAGGACTCCATTT	Acute	+	+	ND	+	+
p38	101757	AGGTGCTCAGGACTCCATTT	Chronic	ND	+ ($p \leq 0.07$)	+	+ ($p \leq 0.06$)	+
STAT 6	195428	CCACAGAGACATGATCTGGG	Acute	ND	+	+	+	ND
STAT 6	195428	CCACAGAGACATGATCTGGG	Rechal.	ND	+	+	+	+
TNF-alpha	25302	AACCCATCGGCTGGCACCAC	Acute	ND	+	+	–	--
TNF-alpha	25302	AACCCATCGGCTGGCACCAC	Chronic	ND	+	+	+	+

+ means response is significantly or nearly significantly different from control. All $p \leq 0.05$ unless otherwise indicated.
– means response is not significantly different from control.
ND means not done.

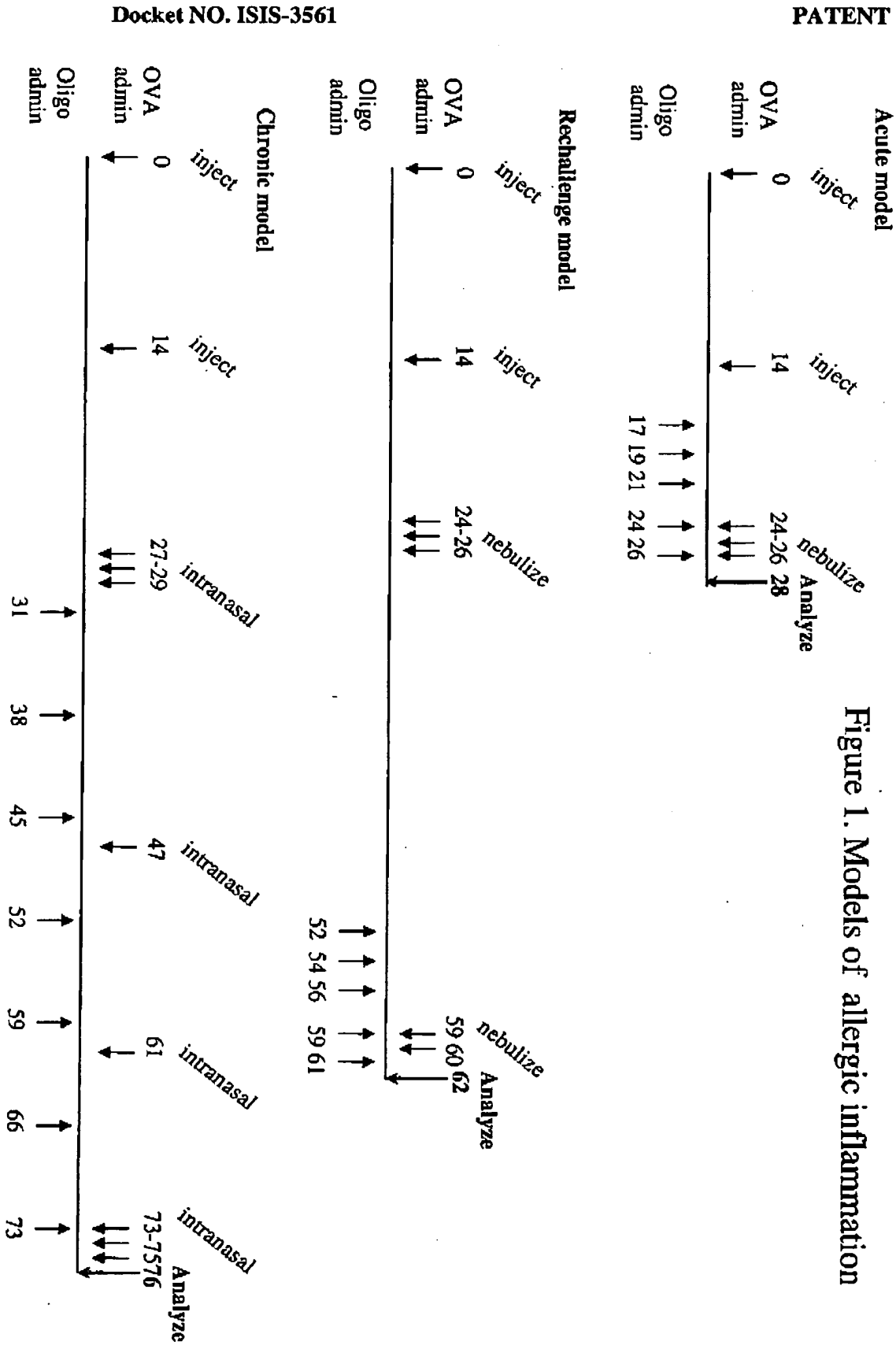

Figure 1. Models of allergic inflammation